Remarks

Claims 1-67, 69-74, 76-81, and 89-107 were pending. By this amendment, claims 1-46, 52, 77-81, and 89-106 are cancelled without prejudice due to the restriction requirement. Claims 108-118 are added. Therefore, claims 47-51, 53-67, 69-74, 76, and 107-118 are now pending.

Support for the claim amendments and new claims can be found throughout the specification, for example:

Claims 47 and 53: claim 52; page 8, lines 29-37; page 14, line 11-page 15, line 2.

Claim 51: rewritten as an independent claim.

Claims 71 and 108: FIGS. 1 and 2, claims 27-29 and 96-97.

Claims 72 and 109: FIG. 1, claims 41-42 and 100 and 101.

Claim 73: FIG. 3; page 5, lines 22-24; and claims 44 and 103-104.

Claim 74: FIG. 3 and page 5, lines 24-27.

Claim 110: FIG. 1 and page 29, lines 35-36.

Claim 111: FIG. 1 and page 4, lines 16-19.

Claim 112: FIG. 5; page 49, lines 7-9; page 51, lines 21-24.

Claims 113-115: page 22, line 31 – page 23, line 1.

Claims 116-118: page 21, lines 1-6, and page 24, lines 9-15.

No new matter is introduced by this amendment, and no amendments are made to distinguish prior art.

Summary of telephone interview

Applicants thank Examiner Slobodyansky for the courtesy of a telephone interview with Applicants' representative Sheree Lynn Rybak, Ph.D. on April 10, 2007. During this interview, the 35 U.S.C. § 112 and 102 rejections were discussed.

With regards to the 35 U.S.C. § 112, first paragraph rejections, Applicants' representative agreed to include a sequence identifier in claim 47 to overcome the written description rejection. With regards to the enablement rejection, Applicants' representative explained that the specification provides teaching for where substitutions will likely be tolerated, and where

substitutions may cause loss of alanine 2,3-aminomutase activity. In addition, Applicants' representative noted that since the filing of this application, numerous other alanine 2,3-aminomutases have been identified. The Examiner agreed to consider such evidence if provided. Applicants' representative agreed to include functional language in claim 47 so that non-functional variants are not claimed. However, agreement was not reached on the percent identity that should be included in the claims. With regards to the rejection of claims 71-74, the Examiner explained that including enzymes and substrates would overcome the enablement rejection of these claims.

With regards to the 35 U.S.C. § 102 rejection, Applicants' representative explained that data in the current specification casts doubt on the results provided in the Frey *et al.* documents cited against the present application. It was noted that Applicants tested two different lysine 2,3-aminomutases, and no alanine 2,3-aminomutase activity was observed in a cell (Tables 2 and 3). However, when 3 or 5 amino acids changes were made (see FIGS. 4 and 5) to two different lysine 2,3-aminomutases, alanine 2,3-aminomutase activity was observed in a cell (Tables 2 and 3). As the *C. subterminale* lysine 2,3-aminomutase of Frey *et al.* is 73% identical to the *P. gingivalis* lysine 2,3-aminomutase tested by the inventors and does not include a change at the amino acid position equivalent to D331/D339 in the disclosed alanine 2,3-aminomutases (see Exhibit A), one skilled in the art would not expect the *C. subterminale* lysine 2,3-aminomutase of Frey *et al.* to have alanine 2,3-aminomutase activity. Applicants' representative also explained that the conditions provided in Frey *et al.* were not performed in a cell; the results were under artificial *in vitro* conditions. The Examiner agreed to reconsider the teachings of Frey *et al.* in view of this information.

Claim objections

Claims 51-56 and 59-64 are objected to on the ground that they recite non-elected sequences (e.g. SEQ ID NO: 20 and 21). Applicants request reconsideration.

Claims 51-54 are amended to remove reference to SEQ ID NO: 21. Claims 59-62 and 64 are amended to remove reference to SEQ ID NO: 20. Claims 55-56 and 63 do not recite a sequence identifier and so these claims are improperly objected to.

In view of these amendments, Applicants request that the objections to the claims be withdrawn.

35 U.S.C. § 112, first paragraph (written description)

Claims 47-50, 57, 58, 65-67, 69-74, 76 and 107 are rejected under 35 U.S.C. § 112, first paragraph as failing to comply with the written description requirement. Applicants disagree and request reconsideration.

Claim 47 has been amended to include a sequence identifier. It is stated on page 5 of the Office action that the specification does not provide any structure:function correlation present in members of the alanine 2,3-aminomutases genus. However, the specification provides guidance on how to identify members of the genus, and where mutations should not be made and where mutations are likely to be tolerated. For example, exemplary fragments and substitutions that can be made to SEQ ID NO: 21 or 30 are provided on page 22, lines 15-20. For example, page 49, lines 7-9 and FIG. 6 explain that a substitution at D331 in *P. gingivalis*/D339 in *B. subtilis* is likely to be important, as a substitution at this equivalent position was found in both sequences. Thus one skilled in the art would expect that the Asp (D) needs to be changed at this residue in an lysine 2,3-aminomutase to obtain alanine 2,3 aminomutase activity. In contrast, residues conserved between the starting lysine 2,3 aminomutase are more likely to tolerate substitution. Therefore, other representative examples of alanine 2,3 aminomutases are provided by the specification.

35 U.S.C. § 112, first paragraph (enablement)

Claims 47-50, 52-58, 60-63, 65-67, 69-74, 76 and 107 are rejected under 35 U.S.C. § 112, first paragraph as failing to comply with the enablement requirement. Applicants disagree and request reconsideration.

Applicants were the first to identify enzymes having alanine 2,3-aminomutase activity. Applicants demonstrated that such activity can occur in a cell. These enzymes convert alpha alanine to beta alanine. These newly identified enzymes permit conversion of alpha alanine to beta alanine *in vivo*.

Due to the restriction requirement, SEQ ID NO: 30 was elected. SEQ ID NO: 30 is an alanine 2,3-aminomutase obtained by mutating a *P. gingivalis* lysine 2,3-aminomutase, and screening for mutants with the desired enzyme activity. The specification provides another alanine 2,3-aminomutase obtained by mutating a *B. subtilis* lysine 2,3-aminomutase (SEQ ID

NO: 21). The sequence identity between these alanine 2,3-aminomutases (SEQ ID NOS: 21 and 30) is 52%.

Claim 47 is amended to recite that the alanine 2,3-aminomutase has at least 90% sequence identity to SEQ ID NO: 30 and that variants retain the ability to convert alpha-alanine to beta-alanine in a cell. Therefore, non-functional variants are not encompassed by the claims. The specification is enabled for the scope of claims with this combination of structural and functional elements. Claim 112 specifies particular residues at position 331, thereby providing further structural elements. Claim 53 recites an alanine 2,3-aminomutase having at least 95% sequence identity to SEQ ID NO: 30, and that variants retain the ability to convert alpha-alanine to beta-alanine in a cell. It is generally the USPTO's position that identifying and selecting sequences having at least 95% sequence identity to a native sequence does not require undue experimentation, and are thus enabled. It is generally asserted on pages 6-7 of the Office action that Applicants have not provided: regions of the protein which can be modified, general tolerance of an alanine 2,3-aminomutase to modification, a rational for identifying which resides in a alanine 2,3-aminomutase to modify to obtain the desired biological activity, and which possible substitutions are likely to be successful. Applicants disagree.

The specification provides teaching on the structure of alanine 2,3-aminomutase enzymes (which includes elected SEQ ID NO: 30). Based on this information, regions of SEQ ID NO: 30 that can be modified and the likelihood of tolerance for modification can be determined by one skilled in the art. Information on the structure of alanine 2,3-aminomutaseis provided in Example 6 (starts on page 48). For example, the FeS cluster-binding motif and putative PLP-binding motifs are indicated (for example see FIG. 4). In addition, the changes to a lysine 2,3-aminomutase that resulted in an alanine 2,3-aminomutase are discussed on page 49, lines 1-12, and shown in FIGS. 4-6. Therefore, based on the structural information provided for alanine 2,3-aminomutase enzymes one skilled in the art can identify regions and residues of SEQ ID NO:3 0 that can be modified while retaining alanine 2,3-aminomutase activity, without undue experimentation.

The specification provides significant teaching on amino acid residues that are conserved and not conserved between alanine 2,3-aminomutase enzymes and provides specific suggestions for modifications. Based on this information, one skilled in the art can identify which resides in SEQ ID NO: 30 can be modified to obtain the desired alanine 2,3-aminomutase activity, and

which modifications are likely to be successful. Methods for selecting and making amino acid substitutions are known in the art. Particular guidance for choosing amino acid modifications to SEQ ID NO: 30, and how such variants can be tested for alanine 2,3-aminomutase biological activity, are provided in the specification.

The specification teaches how to make and select residues of SEQ ID NO: 30 that can be altered, such as substituted or deleted. For example, the specification provides a description of amino acid substitutions that are generally considered conservative (page 11, line 12 – page 12, line 6), and notes that such substitutions generally do not significantly alter the ability of the protein to convert alpha-alanine to beta-alanine. Exemplary substitutions that can be made to SEQ ID NO: 30 are provided (for example see page 22, lines 17-20 and corresponding changes to those described on page 51, lines 22-23 can also be made). In addition to substitution of amino acids, the specification provides that fragments of SEQ ID NO: 30 can be used that retain alanine 2,3-aminomutase activity. It is appreciated that the full-length sequence may not be necessary to retain alanine 2,3-aminomutase activity. Particular exemplary fragments are provided in the specification (for example see page 8, lines 26-27 and page 22, lines 16-17).

In addition to the specific changes provided in the specification, the specification provides teaching that permits one skilled in the art to select other residues that can be modified, with a reasonable expectation of success. An amino acid sequence alignment of the disclosed alanine 2,3-aminomutase enzymes (including SEQ ID NO: 30, Pgaam) is provided in FIG. 6. One skilled in the art will instantly recognize that such an alignment provides information for making amino acid variants.

For example, it is appreciated in the art that changing an amino acid that is conserved at a particular position between the disclosed alanine 2,3-aminomutase enzymes will more likely result in a change in biological activity to the enzyme. In contrast, substituting an amino acid that is not highly conserved between the alanine 2,3-aminomutase enzymes, for example with a conservative amino acid, will not likely significantly alter the biological activity of the enzyme. FIG. 6 also teaches one skilled in the art that deletions can be made to SEQ ID NO: 21 (Bsaam), as it includes several amino acid stretches not found in SEQ ID NO: 30 (Pgaam) (dashes in FIG. 6). This also indicates that insertions could be made to SEQ ID NO: 30 (Pgaam) at these regions, without significant effects on biological activity. In addition, it is appreciated in the art that changing the Asp (D) at position 331/337 (bold in FIG. 6), for example to a Gly or His (or

Gln, Thr, or Asn, see page 51, lines 21-24) will more likely result in an enzyme having alanine 2,3-aminomutase activity, as this Asp was changed in both native lysine 2,3-aminomutase enzymes to produce an enzyme having alanine 2,3-aminomutase activity.

The specification provides methods for determining which variants retain alanine 2,3-aminomutase activity *in vivo*. Alanine 2,3-aminomutase activity and methods of measuring its activity are described on page 8, lines 29-37, and a more thorough description of particular methods is provided in Examples 6 and 9-11. For example, Example 9 (beginning on page 52) provides methods of assaying for the presence of beta-alanine.

Therefore, the specification provides specific examples of substitutions that can be made to SEQ ID NO: 30 and retain alanine 2,3-aminomutase activity, and provides an alignment of alanine 2,3-aminomutase sequences that one skilled in the art can use to identify additional substitutions that can be made while retaining alanine 2,3-aminomutase activity without undue experimentation.

Additional evidence that one skilled in the art can identify alanine 2,3-aminomutases having at least 90% sequence identity to SEQ ID NO: 30 which can convert alpha-alanine to beta-alanine in a cell, is provided in WO 06/047589 and WO 07/047773 (Exhibits B and C). As shown in these PCT publications, numerous variants of SEQ ID NOS: 21 and 30 have been identified and retain alanine 2,3-aminomutase activity. WO 06/047589 discloses alanine 2,3-aminomutase sequences derived from *B. subtilis* lysine 2,3-aminomutase (Bskam), and are at least 90% identical to *B. subtilis* alanine 2,3-aminomutase Bsaam (SEQ ID NO: 21). WO 07/047773 discloses alanine 2,3-aminomutase sequences derived from *P. gingivalis* lysine 2,3-aminomutase (Pgkam), and are at least 90% identical to *P. gingivalis* alanine 2,3-aminomutase Pgaam (SEQ ID NO: 30). Therefore, based on the teachings of the present application, one skilled in the art can generate alanine 2,3-aminomutases having at least 90% sequence identity to SEQ ID NO: 30 that have alanine 2,3-aminomutase in a cell.

Claims 71-74 are amended to clarify the precursors and enzymes present in the cell to obtain the desired product, as requested by the Examiner.

In summary, the specification provides substantial enablement for making and testing variants of SEQ ID NO: 30, and it would not require undue experimentation to identify variant alanine 2,3-aminomutases having the ability to convert alpha-alanine to beta-alanine in a cell.

Therefore, the 35 U.S.C. § 112, first paragraph enablement rejection is improper, and Applicants request that it be withdrawn.

35 U.S.C. § 101

Claim 47 is rejected under 35 U.S.C. § 101 as directed to non-statutory subject matter. Applicants request reconsideration.

As suggested by the Examiner, claim 47 is amended to recite that the peptide is an "isolated" peptide.

In view of this amendment, Applicants request that the 35 U.S.C. § 101 rejection be withdrawn.

35 U.S.C. § 102(a, e)

Claims 47-50, 52, 53, 55-58, 60-63, 65-67, 69-74, 76 and 107 are rejected under 35 U.S.C. § 102(b) as anticipated by Frey *et al.* (US 6,248,874) as evidenced by Frey *et al.* (US 2003/0113882). Applicants disagree and request reconsideration.

The present application discloses for the first time the existence of a polypeptide having alanine 2,3-aminomutase activity *in vivo*. Specifically, Applicants were the first to disclose that mutation of a nucleic acid molecule encoding a lysine 2,3-aminomutase, can provide a nucleic acid molecule that encodes a polypeptide having alanine 2,3-aminomutase activity.

Claim 47 has been amended to clarify that the claimed alanine 2,3-aminomutase enzymes have at least 90% sequence identity to SEQ ID NO: 30 and have the ability to convert alpha-alanine to beta-alanine in a cell. Frey *et al.* do not provide an enzyme having at least 90% sequence identity to SEQ ID NO: 30 and the ability to convert alpha-alanine to beta-alanine in a cell, and therefore does not anticipate the present claims.

There is no disclosure in US 6,248,874 that teaches a skilled artisan that mutating the lysine 2,3-aminomutase isolated form *Clostridium subterminale* would provide an alanine 2,3-aminomutase. Moreover, there is no disclosure in US 2003/0113882 that teaches a skilled artisan that a mutated lysine 2,3-aminomutase from *Clostridium subterminale* would *inherently* function as an alanine 2,3-aminomutase. Accordingly, the combined teachings of US 6,248,874 and US 2003/0113882 does not provide any evidence to demonstrate that a mutated lysine 2,3-

aminomutase would have alanine 2,3-aminomutase activity, thus casting serious doubt of the alleged implicit disclosure of US 6,248,874.

In addition, Applicants bring the Examiner's attention to the unusual *in vitro* conditions which are employed in US 2003/0113882 in order to observe alanine 2,3-aminomutase activity (see paragraphs 151-153 of US 2003/0113882). It is submitted that these conditions could not be reproduced *in vivo*, and therefore such alanine 2,3-aminutase activity would not be observed *in vivo*.

Based on the Applicants' data in the present application with lysine 2,3-aminomutases from *P. gingivalis* and *B. subtilis*, it is Applicants' position that the *Clostridium subterminale* lysine 2,3-aminomutase disclosed in the cited art would not have alanine 2,3-aminomutase activity *in vivo*. The likely inability of the native *Clostridium subterminale* lysine 2,3-aminomutase disclosed in the cited art to have *in vivo* alanine 2,3-aminomutase activity is confirmed by Tables 2 and 3 of the present application, found at pages 50 and 53 respectively. The data contained in Table 2, demonstrates that native lysine 2,3 aminomutase from *P. gingivalis* does not posses alanine 2,3-aminomutase activity. The data contained in Table 3, demonstrates that native lysine 2,3 aminomutase from *B. subtilis* does not posses alanine 2,3-aminomutase activity. However, mutating particular positions of the native *P. gingivalis* and *B. subtilis* lysine 2,3 aminomutase resulted in enzymes with alanine 2,3-aminomutase activity (Tables 2 and 3). It is submitted that the present application teaches that native lysine 2,3-aminomutases do not possess alanine 2,3-aminomutase activity and thus casts further doubt over the alleged implicit disclosure of US 6,248,874. Applicants' request that the Office not ignore the data provided by the Applicants, as it refutes what the cited art arguably teaches.

Further evidence that the native *Clostridium subterminale* lysine 2,3-aminomutase disclosed in the cited art does not have *in vivo* alanine 2,3-aminomutase activity is provided by Exhibit A. Exhibit A is an alignment of *P. gingivalis* lysine 2,3 aminomutase (Pgkam), *P. gingivalis* alanine 2,3-aminomutase (Pgaam), and *Clostridium subterminale* lysine 2,3-aminomutase (Cskam). As shown in this alignment, the amino acid at position 331 (boxed) is identical between the *Clostridium subterminale* lysine 2,3-aminomutase of Frey *et al.* and the native *P. gingivalis* lysine 2,3 aminomutase. As taught by the present specification, changing the amino acid at this position was involved in conferring the ability of a native lysine 2,3-aminomutase enzyme to have alanine 2,3-aminomutase activity (see page 49, lines 7-9). As this

residue is unchanged in the *Clostridium subterminale* lysine 2,3-aminomutaseof Frey *et al.*, one skilled in the art would not expect this enzyme to have alanine 2,3-aminomutase activity *in vivo*.

In summary, based on the data provided in the present specification, the *Clostridium subterminale* lysine 2,3-aminomutase of Frey *et al.* does not have alanine 2,3-aminomutase activity *in vivo*. Because the cited the *Clostridium subterminale* lysine 2,3-aminomutase of Frey *et al.* do not anticipate the claims, the 35 U.S.C. §§ 102(b) and 102(e) rejections are improper, and Applicants request that they be withdrawn.

If there are any minor issues to be resolved before a Notice of Allowance is issued, the Examiner is invited to telephone the undersigned.

Respectfully submitted,

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